

Project Report: Molecular survey of extremophile microbial diversity in hypersaline ecosystems

**Project Investigator:**

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Project Progress

Projects continue to revolve around the development and use of rRNA-based molecular methods to study the microbial constituents of ecosystems in extreme environments without the requirement for cultivation of the organisms. This cultivation-independent approach to ecosystems analysis is essential rather than classic methods because most microbes, >99%, are not cultured using standard techniques. With the molecular methods, rRNA genes are cloned directly from environmental DNA, and then sequenced to gain a phylogenetic snapshot of the organisms represented by the cloned genes. Some properties of organisms can be inferred from the phylogenetic results, and the sequences can be used to design hybridization probes to visualize organisms and their interactions in the environment. Recent results in NAI sponsored studies include:

1. Antarctic and Colorado endolithic communities. Primary productivity in rocks occurs through the action of endolithic microbial communities, photosynthesis-driven communities in the outer few cm of any rock surface exposed to light. These communities so far have only received limited study, only with classic microscopy and culture techniques. Ongoing rRNA gene analyses of Antarctic and Rocky Mountain endolithic communities have revealed many novel kinds of organisms, some closely related to described organisms, but others very different. A particularly noteworthy discovery this past year has been a highly acidic (pH 1) endolith community in a sinter field at Yellowstone. Molecular analyses showed the main community consists of cells of *Cyanidium caldarum*, a eukaryotic alga, embedded in a dense mesh of previously unknown *Mycobacterium* spp. The communities fossilize readily, encouraging their consideration as a target in the search for life on Mars. (Manuscript submitted to Science.)
2. Yellowstone high-temperature settings: This laboratory has for many years studied thermophilic ecosystems at Yellowstone and elsewhere. Current activities continue to explore the makeup of properties of communities that, during the past year, we have shown to depend on metabolism of hydrogen as an energy source. (Manuscript in preparation for PNAS.)

3. Hypersaline ecosystems: We are engaged in extensive analysis of the microbial composition of Guerrero Negro hypersaline mats, in concert with geochemical and other biological studies of the NAI Ecogenomics group. Particularly noteworthy at this stage is the finding, based on rRNA gene abundance, that so-called Green Nonsulfur Bacteria, not cyanoacteria, are the dominant species. This poses many hypotheses and new questions.

Overall, we are making excellent progress and this work is scientifically conspicuous. Additionally, the PI participates in numerous astrobiology-related public and institutional activities, for instance participation as a plenary speaker at the 2004 NAI Annual Meeting.

### Highlights

- We have shown by phylogenetic, geochemical and thermodynamic measurements and modeling that Yellowstone and probably other geothermal ecosystems depend on hydrogen as energy source.
- Studies of Guerrero Negro hypersaline microbial mats showed that Green Nonsulfur Bacteria, not Cyanobacteria, dominate hypersaline microbial mats. This has impact on expected mechanisms of carbon fixation, isotope fractionation and other geochemical parameters.
- The ongoing Guerrero Negro studies have discovered and identified by sequence >7000 novel microbes, some only distantly related to known organisms. The number of bacterial kingdoms was expanded from about 60 to about 80 in the study. (This is major!)
- The newly discovered acidic Yellowstone endolithic community is an entirely novel biome with relevance to the possibility of life on Mars and to the search for past life there.

### Roadmap Objectives

- **Objective No. 3.4:** Origins of cellularity and protobiological systems
- **Objective No. 4.1:** Earth's early biosphere
- **Objective No. 4.2:** Foundations of complex life
- **Objective No. 5.1:** Environment-dependent, molecular evolution in microorganisms
- **Objective No. 5.2:** Co-evolution of microbial communities
- **Objective No. 5.3:** Biochemical adaptation to extreme environments
- **Objective No. 6.1:** Environmental changes and the cycling of elements by the biota, communities, and ecosystems
- **Objective No. 7.1:** Biosignatures to be sought in Solar System materials
- **Objective No. 7.2:** Biosignatures to be sought in nearby planetary systems